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Headspace solid-phase microextraction profiling of volatile compounds in urine: application to metabolic investigations

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Abstract

Volatile compounds contribute substantially to the metabolic pool in man. Their analysis in body fluids is problematic. We investigated headspace solid-phase microextraction (HS-SPME) with Carboxen–polydimethylsiloxane fibres and gas chromatography–mass spectrometry for profiling urinary volatile components. These fibres were more sensitive for very volatile and sulfur compounds than three other phases tested. We detected a wide range of compounds in normal urine at acid and alkaline pH. Profiles presented for five individuals with metabolic disturbances demonstrate abnormal accumulation of sulfur compounds, fatty acids and plasticisers. HS-SPME can complement profiling of non-volatile compounds in metabolic investigations and could be a useful extension of the diagnostic repertoire.

Keywords: Headspace solid-phase microextraction; Metabolism; Volatile compounds

1. Introduction

Volatile organic compounds have a boiling point below 300°C and generally less than 12 carbon atoms [1,2]. They make a substantial contribution to the metabolic pool in man. Sources include food, food additives or contaminants, pollutants in air or in medical devices introduced into the body, bacterial fermentation in the large bowel, and metabolic processes in the body [1,3–5]. They are chemically very diverse. Those found in urine and plasma include alcohols, aldehydes, furans, ketones, pyrroles, terpenes and other heterocyclic compounds [1–3,6].

Multicomponent analysis (metabolic profiling) has contributed considerably to our understanding of the metabolism of *non-volatile* organic compounds in man, notably organic acids [7]. Urine has been the preferred biological fluid since compounds are concentrated by the kidney before excretion. Urine profiling is much more difficult for *volatile* compounds because of their volatility, structural diversity and differences in their polarity and concentrations [1]. Currently, capillary gas chromatography (GC) provides the best resolution of urine components with coupling to mass spectrometry (MS) for positive identification. Analytical methods have included direct injection of urine [8] or headspace (HS) vapour [1] onto a GC column and solvent extraction [9,10]. However, these methods have poor sensitivity, and sample losses and contamination are risks

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with solvent procedures. Steam distillation is an alternative [11]. Preferred procedures involve sparging the urine with an inert gas and concentrating the stripped volatile compounds on a cryogenic trap or by adsorption onto a porous polymer (usually Tenax) before desorption into the GC system [1,2,4,6,12–15]. Transfer into the gas phase is favoured by addition of salt, stirring and raising the temperature [1]. Transevaporator sampling combines HS or solvent extraction with Tenax trapping [1,16].

No single analysis can provide a profile that represents the true concentrations of components in urine because differences in their volatility and chemical properties influence extraction. However, invaluable data has been obtained by research groups for healthy human subjects [4,6,12,13], patients with diabetes [1,12] and liver and kidney disease [6], and for normal, starved and diabetic animals [3,15,17,18]. Selected volatile components have been analysed in urine of patients with inherited disorders of branched-chain amino acids [8,10,19,20]. To extend our incomplete knowledge of these compounds in health and disease we need a simpler analytical procedure that does not require dedicated equipment and which can be used in parallel with other diagnostic analyses in clinical laboratories.

Solid-phase microextraction (SPME) was introduced a decade ago by Arthur and Pawliszyn [21] as a rapid extraction technique for the analysis of volatile and semi-volatile compounds from a variety of matrices. The method uses a modified syringe assembly that houses a short fused-silica micro-fibre externally coated with a stationary phase. A range of phases is available for different applications [22,23]. The technique involves either the equilibrium or non-equilibrium partitioning of analytes between the stationary phase and sample, followed by desorption of the analytes in the hot injector of a GC system. SPME can be operated in two modes, either HS sampling or with immersion directly into the sample [22]. HS sampling is preferred for biological fluids. SPME has been used to analyse blood and urine for a range of drugs and their metabolites, volatile anaesthetic gases and solvents [22–24]. So far its value for investigating metabolism has been barely explored. Reports are limited to analysis of trimethylamine [25], organic acids [26] and steroids [27] in urine, volatile compounds in blood [28], volatile fatty acids in faeces [29] and ketone bodies in breath [30].

The aim of this study was to explore the potential of SPME for profiling volatile organic components of normal urine and in illnesses associated with disturbances of volatile compounds. We selected Carboxen–PDMS fibres for the study, but have investigated other fibres. The examples shown indicate the versatility of the method, and highlight our ignorance of the metabolism of volatile compounds.

2. Experimental

2.1. Urine samples and materials

Random urine samples were collected from 10 healthy adult volunteers on a mixed diet. Urine specimens from patients with suspected metabolic disorders were random samples collected for diagnostic biochemical analyses. Urine used to compare different SPME fibres and incubation conditions was obtained with informed consent from a 40-year-old woman with cirrhosis and insulin dependent diabetes mellitus. All specimens were collected into 20 ml sterile PVC containers. Samples were analysed fresh, or frozen immediately and stored at −20°C.

Polydimethylsiloxane (PDMS) (100 μm film thickness), polyacrylate (PA) (85 μm film thickness), Carbowax–divinylbenzene (CW–DVB) (65 μm film thickness) and Carboxen–PDMS (75 μm film thickness) SPME fibres and SPME fibre syringe holders were from Supelco (Poole, UK). HS vials (22 ml) with soft silicone rubber seals (20 mm diameter) and aluminium caps were from Alltech Associates (Carnforth, Lancashire, UK). Analytical-grade chemicals and reagents were from Sigma–Aldrich (Gillingham, UK) or Fisons (Loughborough, UK). Water was deionised by reverse osmosis.

2.2. Instrumentation

The bench-top GC–MS system was a 5890 series 2 GC instrument linked to a 5971A quadrupole MS system (Hewlett-Packard, Bracknell, UK) fitted with a BP-20 fused-silica capillary column (30 m×0.25 mm I.D., film thickness 0.25 μm) from Scientific Glass Engineering (Milton Keynes, UK). Helium was used as the carrier gas at a flow-rate of 1 ml/min. A narrow bore (0.75 mm) SPME injection liner was used (Supelco). The GC–MS system was

operated under the following conditions: no solvent delay; injector 250°C; interface transfer line 280°C; oven temperature programme 40°C (5 min) then 10°C/min to 220°C (10 min). The MS system was operated in the scan mode from 34 to 300 amu. The detector signals were collected, integrated and recorded using a HP Chemstation (Hewlett-Packard). Compounds were identified with reference to authentic standards and/or the Wiley mass spectral library.

2.3. Sample preparation and SPME procedure

All SPME fibres were pre-conditioned by inserting them into the GC injector according to the manufacturer's instructions. To prevent surface adsorption of analytes, all glassware, HS vials and magnetic stirrers were silanised for 1 h in a solution of dichlorodimethylsilane (approximately 10%, v/v, in cyclohexane), thoroughly washed with methanol and oven dried prior to use.

Three different sample preparation procedures were used. (1) No pH adjustment (pH 5.0–7.0): 4 ml of urine, 1 ml of water and approximately 3 g of sodium chloride were added to the HS vial. (2) Acid conditions (pH 1–2): 4 ml of urine, 1 ml of water, approximately 3 g of sodium chloride and 100 μl of 6 *M* HCl were added to the HS vial. (3) Alkali conditions (pH 12–14): 4 ml of urine, 1 ml of water, approximately 3 g of potassium carbonate and one pellet of KOH were added to the HS vial. A stirring bar was added and each vial quickly crimp-sealed with a silicone rubber septum.

Urine from a child with the inherited metabolic disorder medium-chain acyl-CoA dehydrogenase (MCAD) deficiency was subjected to a separate alkaline hydrolysis experiment in order to liberate fatty acids from glycine, glucuronide and carnitine conjugates [31,32]. A 4 ml volume of urine, approximately 3 g of sodium chloride, two pellets of KOH and a stirring bar were added to the HS vial which was sealed and placed in a heating block at 80°C for 1 h. The pH of the sample at the start of the procedure was 14. After incubation, the vial was cooled to room temperature. The hydrolysed sample was adjusted back to pH 1–2 by the addition of 250 μl of 6 *M* HCl added directly through the septum using a 1 ml hypodermic syringe.

Vials were mounted on a magnetic stirrer submerged in a water bath maintained at 50°C, and the contents stirred continuously so as to release volatile compounds into the HS. The septum of the sample vial was pierced with the SPME needle guide and the SPME fibre exposed to the HS vapour for 30 min. The extracted compounds were then desorbed (2 min) from the fibre in the GC injector port, split valve closed for 2 min. To ensure no carry over of extracted material during analyses, the SPME fibres were further conditioned between runs for 6 min in a hot injector of a separate GC (260 to 290°C depending on fibre chemistry) operating with a high split flow of helium carrier gas. This additional step is advised with the Carboxen–PDMS fibre because some larger-molecular-mass analytes may condense deep inside the pores of the Carboxen 1006 phase, and can only be removed effectively by high desorption temperatures [23].

3. Results and discussion

3.1. Instrumental conditions

The polar (BP-20) GC stationary phase gave good peak shapes for a wide range of different analytes extracted by SPME fibres. The GC conditions were adjusted so as to achieve good separation within a reasonable sample throughput time. At an initial oven temperature of 40°C, acceptable peak shapes of volatile early eluting analytes (which included gases at room temperature) were achieved and there was no need for cryo-focusing. With a final oven temperature of 220°C all of the extracted compounds were eluted from the column. Consecutive analyses of samples and blanks confirmed that there was no carry over of extracted material with any of the different fibres used.

3.2. Sample pH and choice of SPME fibres

A range of fibre coatings is available for SPME, classed by polarity and film thickness. PDMS is non-polar and PA and Carbowax more polar. Blending the phases with porous particles (DVB or Carboxen 1006) improves sensitivity for some compounds. The pore size of Carboxen–PDMS (2–20 Å) is ideal for trapping small molecules and its high porosity provides a large surface area. The pores pass through the phase particles facilitating rapid

desorption [23]. These fibres have high sensitivity for volatile acids and alcohols ($<C_8$) (10 ppt–1 ppm), C_2–C_8 aldehydes (1–500 ppb) and C_3–C_9 ketones (5 ppb–1 ppm) [23,33] and have been used to analyse sulfur compounds [34,35]. Many of the volatile components of urine fall into these chemical groups.

In order to assess the effects of different sample preparation and extraction procedures, aliquots of one urine sample, from a patient with cirrhosis and diabetes mellitus, were analysed to compare the range of compounds extracted by four fibre types at different pH: without pH adjustment (pH 6.8), acid and alkaline pH. All analyses were carried out with 30 min extraction times at 50°C. Few compounds were extracted by any of the fibres at pH 6.8 and peak areas were small. Under acid conditions sharp, well resolved peaks were obtained with the Carboxen–PDMS fibre for very volatile compounds such as acetone (propanone), butanone and dimethylsulfide but they were extracted poorly by the PDMS, PA and CW–DVB fibres. These fibres performed better for less volatile compounds such as food flavourings and additives. The CW–DVB fibre was best for extracting medium-chain (C_8 to C_{12}) carboxylic acids. A different range of compounds was extracted at alkaline pH. Again the Carboxen–PDMS fibre was the most sensitive for the very volatile compounds such as acetone and trimethylamine.

Choice of fibre type and pH of extraction will, therefore, influence the profiles obtained. The pH of incubation and fibre type may be selected according to the urine constituents of interest. The most representative profile of a range of components will be obtained by sampling at acid and alkaline pH with two or more fibres. This is feasible because of the simplicity of the procedure and relatively small sample requirement. For this study, we selected Carboxen–PDMS fibres because of their broad specificity and sensitivity.

3.3. SPME sample extraction temperature and sampling time

Using an extraction time of 30 min with 4 ml aliquots of the same urine sample, the effect of extraction temperature at 30, 40, 50, 60 and 70°C was investigated. The volatile components were extracted under acidic (pH 1–2) conditions using only the Carboxen–PDMS fibre. Raising the temperature progressively increased the number of peaks in the profile. With the same integration parameters, 13, 20, 29, 43 and 48 separate peaks were measured at 30, 40, 50, 60 and 70°C, respectively. This was attributed to larger peak areas (i.e., increased sensitivity) at higher temperature and not to the elution of additional compounds with higher boiling points. Using the volatile sulfur compound dimethyldisulfide as a marker, the largest peak area was obtained at 50°C, with four times the signal obtained at 30°C. The peak area decreased at higher temperatures, and at 70°C was half that at 50°C. The signal for acetic acid, on the other hand, did not change significantly over the range of temperatures tested. Although the greatest number of peaks was obtained at 70°C, we found previously that at temperatures above 50°C with acidic conditions, stripping of the polymer fibre coating from the inner core occurred sometimes [29]. With higher extraction temperatures, the Carboxen–PDMS fibre coating often powdered, swelled and fragmented when it was withdrawn back into the needle guide. An extraction temperature of 50°C was selected since good peak areas were obtained and there is a lower risk of damaging the fibre.

The effect of sampling time at 50°C was investigated for the Carboxen–PDMS fibre using the same urine sample and extraction conditions as above. With the same integration parameters, 23, 27, 29 and 45 separate peaks were recorded for incubation times of 10, 20, 30 and 60 min, respectively. The peak areas of dimethyldisulfide and acetic acid, used as indicators, increased with time to 30 min. The signal for dimethyldisulfide at 30 min was approximately twice that at 10 min and for acetic acid approximately three times greater. However, at 60 min the signal for dimethyldisulfide was only 90% of that at 30 min.

The objective was to profile a range of urinary components with differing physicochemical properties. It is impossible to achieve conditions which will extract them all optimally, simultaneously. Increasing both extraction time and temperature can lead to desorption of some components from the fibre as new equilibrium conditions are achieved. A compromise is necessary. For our clinical inves-

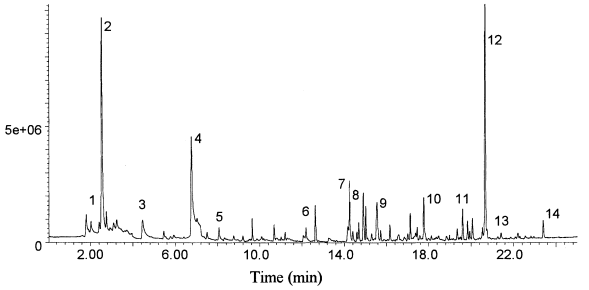

Fig. 1. Total ion current profile of volatile compounds extracted at acid pH from urine of a healthy adult on a normal mixed diet. Urine was saturated with NaCl and acidified with HCl to pH 1–2. A 75 μm Carboxen–PDMS fibre was exposed to the HS vapour for 30 min at 50°C and desorbed for 2 min. Key: (1) methanethiol, (2) acetone, (3) 2-pentanone, (4) dimethyldisulfide, (5) 4-heptanone, (6) 2-methylmercaptofuran, (7) *trans*-linalol oxide, (8) 2-ethylhexanol, (9) vitispirane, (10) 1-α-terpineol, (11) *p*-cymen-8-ol, (12) 2-ethylhexanoic acid, (13) phenol, (14) epoxy-butylated hydroxytoluene.

tigations, we selected an extraction time of 30 min and temperature of 50°C.

3.4. Profiles of volatile compounds in normal urine samples

Ten normal urine samples were analysed at acid and alkaline pH. Representative profiles are shown in Figs. 1 and 2, respectively. The profiles differed under acidic and alkaline conditions. Acids and

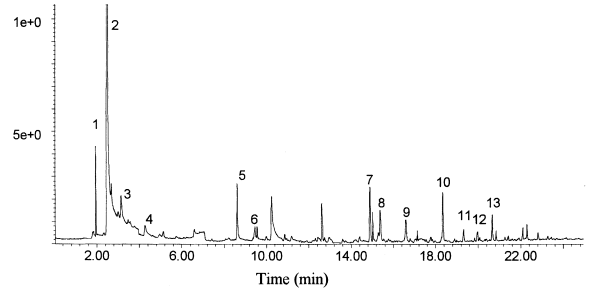

Fig. 2. Total ion current profile of volatile compounds extracted at alkaline pH from urine of a healthy adult on a normal mixed diet. Urine was saturated with K_2CO_3 and alkalinised with KOH to pH 12–14 and analysed using conditions as described in Fig. 1. Key: (1) trimethylamine, (2) acetone, (3) 2-butanone, (4) 2-pentanone, (5) 1-butanol, (6) pyridine, (7) 2-ethylhexanol, (8) 1H-pyrrole, (9) seudenone, (10) car-2-en-4-one, (11) 1-methyl-2-piperidinone, (12) benzylalcohol, (13) 2-ethylhexanoic acid.

sulfur-containing molecules were extracted better at acid pH. Alkaline pH favoured extraction of alcohols, amines, ketones and *N*-heterocyclic compounds. Table 1 lists the compounds found at both pH levels for all 10 samples. They are structurally very heterogeneous.

The range of substances is comparable with those reported for normal subjects using a variety of purge-and-trap procedures [1,4,12] and for normal and starved rats [15] and normal and diabetic mice [18]. Like others [1,12], we noted variation in the range and concentrations of compounds excreted among individuals. Many components were food additives. Some solvents may have been introduced as laboratory contaminants. As in the other reports, we found a large series of ketones which are probably decarboxylation products of corresponding oxo-acids produced in the urine or as analytical artefacts [3]. The source of the precursor oxo-acids is uncertain. Some are probably products of bacterial metabolism in the colon, since the ketones were found in much higher concentration in urine of conventional than germ-free rats [14].

One ketone, 4-heptanone, was found in low concentration in most normal samples, often with a small peak of 2-heptanone. Its origin is unknown, but it is probably from an exogenous source [6]. It has been reported as a metabolite of 2-ethylhexanol in rats [36], a solvent used in the manufacture of plasticisers and also released by hydrolysis from bis(2-ethylhexyl)phthalate and bis(2-ethylhexyl)adipate, plasticisers added to PVC to make it flexible [36–38]. We have evidence (to be reported separately) that it is produced from the in vivo metabolism of plasticisers in man. Sources for normal subjects would include food contamination by PVC contact films [39]. 2-Ethylhexanol and 2-ethylhexanoic acid were also found universally in our samples. We found that these were introduced as contaminants from the PVC containers used for urine storage.

3.5. Clinical studies

We analysed urine from patients with metabolic disorders with predicted accumulation of volatile compounds. Cases have been selected to show the versatility of the method.

Table 1
Compounds identified in urine from 10 healthy adults on a normal mixed diet analysed at acid pH (1–2) and alkaline pH (12–14)[a]

Acids	Aldehydes and alcohols	Amines	Food	Ketones	*N*-Hetero	*O*-Hetero	Solvents and contaminants	Sulfur compounds
Acetic	Benzaldehyde	Dimethylamine	Benzoic acid	Acetone	2-Cyano-2-butene	2-Acetylfuran	2-Butoxyethanol	Dimethyldisulfide
Acetic acid ethyl ester	Benzylalcohol	Trimethylamine	Car-2-en-4-one	2-Butanone	2,5-Dimethylpyrazine	2,5-Dimethylfuran	Chloroform	Dimethyltrisulfide
n-Butyric	1-Butanol		1-Carveol	3-Dimethyl-2-cyclo-pentene-1-one	1-Me-2-piperidinone	2-Ethyl-5-mefuran	1-Ethyl-2,3-dimethylbenzene	1,3-Dithiacyclohexene
Butyric acid butyl ester	*n*-Hexanal		3-Carvo-menthenone		3-Mepyridine	2-Furanmethanol	2-Ethylhexanoic acid	3-Isothiocyanato-1-propene
Formic	1-Hexanol		Isocineole	2-Heptanone	Me-1-pyrrole	2-Mefuran	2-Ethylhexanol	5-Meisothiazole
Isovaleric/2-mebutyric	3-Me-3-buten-1-ol		*p*-Cresol	4-Heptanone	Mepyrazine		Styrene	Me-2-propenyl-disulfide
n-Nonanoic	5-Me-3-hexanol		Cuminylalcohol	3-Hexanone	Nicotine		Thymol	Me-propyldisulfide
n-Octanoic	1-Octanol		Cymenene	3-Mecyclohexanone	Piperidine		Toluene	Methanethiol
	Phenol		*p*-Cymene-8-ol	2-Me-2-cyclopentene-1-one	Pyrazine		Xylene	2-Methylmercaptofuran
			β-Damascenone	3-Me-2-pentanone	Pyridine			
			Dihydromyrcenol	4-Me-2-pentanone	1H-Pyrrole			
			trans-Edulan	4-Me-3-pentene-2-one	2-Vinylpyrazine			
			Epoxy-butylated hydroxytoluene	2-Pentanone				
			Eucarvone					
			Furan					
			β-Ionone					
			α-Isophorone					
			Isopropyltoluene					
			Linalool					
			trans-Linalol oxide					
			Linaloyl oxide					
			Megastigmatrienone					
			Menthol					
			Phenol					
			Pinane					
			β-Pinene					
			Pulegone					
			Santene					
			Seudenone					
			α-Terpineol					
			1-α-Terpinene					
			γ-Terpinene					
			Terpinene-1-ol					
			4-Terpinol					
			Vitispirane					

[a] Extraction and analytical conditions as described in Fig. 1 (acid) and Fig. 2 (alkaline). Key: me=methyl.

3.5.1. Case 1: Severe fasting ketoacidosis

A 5 year old boy was admitted acutely with drowsiness, dehydration and severe ketoacidosis after vomiting repeatedly for 24 h. Surgical and likely inherited metabolic disorders were excluded. Fig. 3 shows the profile of his alkalinised urine. The major peak was acetone, the decarboxylation product of acetoacetic acid. The origin of the unsaturated ketones which were also present [3-pentene-2-one, 3-hexene-2-one (very large) and 3-heptene-2-one] is unknown. They are probably decarboxylation products of the corresponding unsaturated oxo-acids. 3-Pentene-2-one was found in urine of starved [15] and diabetic rats [3], but 3-hexene-2-one has not been reported to our knowledge. We have preliminary evidence that it may be produced via condensation of propanal with acetoacetate. Further studies to confirm this are underway.

3.5.2. Case 2: Homocystinuria (McKusick 23620)

In this disorder an inherited deficiency of the enzyme cystathionine β-synthase [EC 4.2.1.22] prevents catabolism of methionine by *trans*-sulfuration. Homocysteine and methionine accumulate. Treatment includes betaine to reduce the plasma homocysteine (by methylation to methionine) to prevent thromboses [40]. The accumulating methionine is channelled through a minor transamination pathway shown to produce methanethiol and other sulfides [41]. Accumulation of these compounds would be anticipated in homocystinuria during betaine treatment. Fig. 4 shows the profile of acidified urine from a 37 year old man with homocystinuria, treated with betaine, who was well. His plasma methionine was 1115 μmol/l (reference range<95 μmol/l). An enormous peak of dimethyldisulfide is accompanied by large peaks of methanethiol and 2-methylmercaptofuran and low concentrations of other sulfur compounds. A similar profile was obtained for his 34-year-old brother, also treated with betaine for homocystinuria.

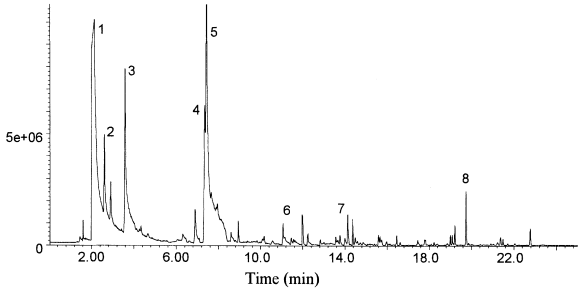

Fig. 3. Total ion current profile of volatile compounds extracted at alkaline pH from urine of a 5 year old child with severe ketosis (case 1). Analytical conditions were as described in Fig. 1. Key: (1) acetone, (2) 2-butanone, (3) 2-pentanone, (4) 3-penten-2-one, (5) 3-hexene-2-one, (6) 3-heptene-2-one, (7) 2-ethylhexanol, (8) 2-ethylhexanoic acid.

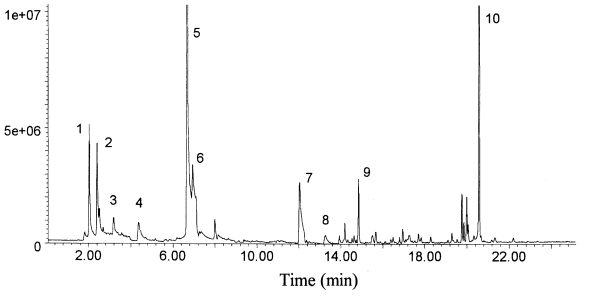

Fig. 4. Total ion current profile of volatile compounds extracted at acid pH from urine of a 37 year old man with homocystinuria (case 2). Analytical conditions were as described in Fig. 1. Key: (1) methanethiol, (2) furan, (3) 2-butanone, (4) 2-pentanone, (5) dimethyldisulfide, (6) hexanal, (7) 2-methylmercaptofuran, (8) dimethyltrisulfide, (9) 2-ethylhexanol, (10) 2-ethylhexanoic acid.

3.5.3. Case 3: Decompensated alcoholic hepatitis

Fig. 5 is the profile of acidified urine from a 63 year old woman with alcohol-induced liver failure. She was receiving fluids intravenously. Dimethyldisulfide dominates the profile, and there are

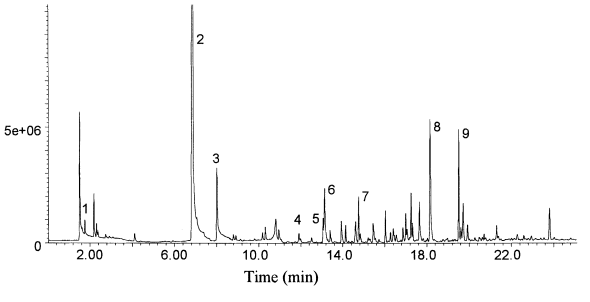

Fig. 5. Total ion current profile of volatile compounds extracted at acid pH from urine of a 63 year old woman with liver failure (case 3). Analytical conditions were as described in Fig. 1. Key: (1) methanethiol, (2) dimethyldisulfide, (3) 4-heptanone, (4) 2-methylmercaptofuran, (5) dimethyltrisulfide, (6) 2-methyl-5-methylthiofuran, (7) 2-ethylhexanol, (8) 3-carvomenthenone, (9) *p*-cymen-8-ol.

smaller peaks of other sulfur compounds. These increases were probably due partly to impaired methionine catabolism through the *trans*-sulfuration pathway because of the liver damage, with diversion through the transamination pathway (see above). In addition, decreased clearance of sulfur compounds absorbed from the intestine from food [42] and colonic bacterial metabolism [43–45] would be contributory.

Analysis of volatile sulfur compounds is difficult. In addition to their volatility, they adsorb to surfaces readily, and may undergo partial oxidation or chemical changes during analysis, especially with lengthy extraction procedures [46,47]. Mestres et al. used SPME with PDMS and PA [48] and Carboxen–PDMS [34] fibres to analyse sulfides, disulfides and thiophene in the HS of wine. Carboxen–PDMS fibres were best, with detection limits of 0.05–4.0 μg/l and recoveries close to 100%. We are unaware of reported applications of SPME for sulfur compounds in biological fluids or breath.

The large increase in urinary 4-heptanone found in case 3 could be explained by increased exposure to 2-ethylhexanol from the intravenous infusion [5,38].

3.5.4. Case 4: Multiple acyl-CoA dehydrogenase deficiency (MADD) [Glutaric aciduria Type II (McKusick 23168)]

In this disorder, at least nine flavine dehydrogenases are inactivated because of an inherited deficiency of electron transfer flavoprotein (ETF) or ETF-coenzyme Q oxido-reductase [49]. A host of metabolites accumulates, including volatile short-chain carboxylic acids from branched-chain amino acids and fatty acids. These contribute to metabolic acidosis and cause an unpleasant body odour. Urine was collected on the fourth (and last) day of life from an acidotic term newborn baby with the most severe form of the disorder. He received intensive medical care which included parenteral nutrition, peritoneal dialysis and haemofiltration and he was therefore exposed to plasticisers from multiple sources. Fig. 6 is the chromatogram of acidified urine. There are large peaks of short-chain fatty acids and the profile is consistent with the diagnosis. A number of short-chain fatty acid esters eluted after 22 min which are not known endogenous metabolites. They may have been analytical artefacts or

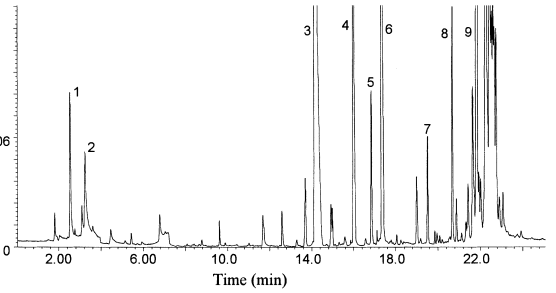

Fig. 6. Total ion current profile of volatile compounds extracted at acid pH from urine of a 4 day old baby with multiple acyl-CoA dehydrogenase deficiency (case 4). Analytical conditions were as described in Fig. 1. Key: (1) acetone, (2) 2-butanone, (3) acetic acid, (4) isobutyric acid, (5) *n*-butyric acid, (6) isovaleric acid, (7) *n*-hexanoic acid, (8) 2-ethylhexanoic acid, (9) a mixture of fatty acid esters (probable artefacts).

formed during sample storage. The profile of alkalinised urine (Fig. 7) is strikingly different. Only small amounts of short-chain acids were extracted and the profile is dominated by very large peaks of the plasticisers cyclohexanone, cyclohexanol and 2-(2-butoxyethoxy)ethanol (butyl carbitol). Cyclohexanone is a solvent sealer for PVC used in many medical devices, including dialysis tubing [5,50]. It is reduced to cyclohexanol in vivo [51]. It is of interest that very little 4-heptanone was found in this baby's urine. Because of his metabolic defect he would be unable to produce this compound from 2-ethylhexanol via the β-oxidation pathway [36].

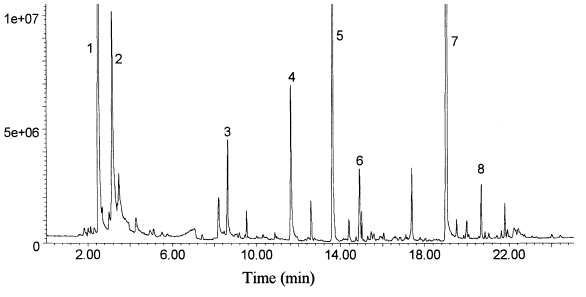

Fig. 7. Total ion current profile of volatile compounds extracted at alkaline pH from urine of a 4 day old baby with multiple acyl-CoA dehydrogenase deficiency (case 4). Analytical conditions were as described in Fig. 1. Key: (1) acetone, (2) 2-butanone, (3) 1-butanol, (4) cyclohexanone, (5) cyclohexanol, (6) 2-ethylhexanol, (7) 2-(2-butoxyethoxy)ethanol, (8) 2-ethylhexanoic acid.

3.5.5. Case 5: Medium-chain acyl-CoA dehydrogenase deficiency (McKusick 201450)

This is an inherited disorder of fatty acid oxidation which may cause hypoglycaemia, brain swelling and death. C_6–C_{12} fatty acids and their metabolites, and carnitine, glucuronide and glycine conjugates accumulate [52]. Fig. 8a shows the profile of acidified urine collected from a 4 year old boy on the day of admission following a respiratory arrest at home. There are small peaks of *n*-hexanoic acid, *n*-octanoic acid and 5-hydroxyhexanoic lactone which we do not find in urine of normal children. After alkaline incubation to hydrolyse glycine and carnitine conjugates (Fig. 8b), there were large increases in *n*-hexanoic and *n*-octanoic acids and small peaks of a series of methyl acids appeared: 4-methylpentanoic (isocaproic), 4-methylhexanoic and 5-methylhexanoic acids.

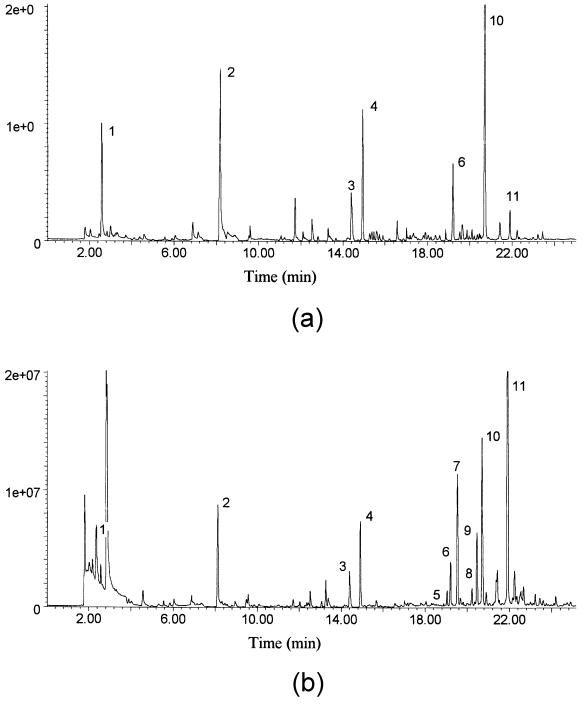

Fig. 8. Total ion current profiles of volatile compounds from urine of a 4 year old child with medium-chain acyl-CoA dehydrogenase deficiency (case 5). Analytical conditions were as described in Fig. 1. (a) Extracted at pH 1–2, (b) after alkaline hydrolysis and re-acidification to pH 1–2. Key: (1) acetone, (2) 4-heptanone, (3) acetic acid, (4) 2-ethylhexanol, (5) 4-methylpentanoic acid, (6) 5-hydroxyhexanoic lactone, (7) *n*-hexanoic acid, (8) 5-methylhexanoic acid, (9) 4-methylhexanoic acid, (10) 2-ethylhexanoic acid, (11) *n*-octanoic acid.

Volatile carboxylic acids accumulate abnormally in inherited defects of branched-chain amino acids and of short- and medium-chain fatty acid oxidation. Some are detoxified in vivo by conjugation with carnitine, glucuronic acid or glycine. Metabolic laboratories diagnose these disorders using GC–MS to detect the glycine conjugates and other, non-volatile, organic acids increased in the diseases, and tandem MS to detect acylcarnitines in body fluids. Few have facilities to analyse the free volatile acids, yet these contribute to the clinical problems. There are relatively few reports of volatile acid profiles in these disorders [8,10,19,20]. With acidic extraction, we found all the diagnostic volatile carboxylic acids in urine from a baby with MADD (case 4). Medium-chain carboxylic acids were found in acidified urine from the patient with MCAD deficiency (case 5) but were not diagnostic. However, the profile was clearly abnormal after alkaline hydrolysis of conjugates. The appearance of methylcarboxylic acids was interesting. 4-Methylpentanoic and 4-methylhexanoic glycine conjugates were identified in urine in MCAD deficiency [31]. Carnitine conjugates of these acids and of 5-methylpentanoic acid have also been identified in urine after hydrolysis and derivatisation [32]. They may be metabolites of branched-chain fatty acids (e.g., phytanic acid) and are specific for MCAD deficiency [31]. There are no reports yet of their identification by tandem MS [32].

4. Conclusion

The five cases presented demonstrate that HS-SPME analysis of urine can complement profiling of non-volatile compounds (e.g., organic and amino acids) in investigations of metabolic disturbances. Furthermore, it can be used to profile other biological matrices (e.g., blood and faeces [29]) offering a more comprehensive overview of intermediary metabolism. HS-SPME is versatile, simple to carry out and does not require special equipment other than a GC–MS system, which is standard for metabolic laboratories. Its introduction into clinical analysis would expand our knowledge of the metabolism

of volatile compounds, which is currently very incomplete.

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